

# Forms and Prevalence of Intersexuality and Effects of Environmental Contaminants on Sexuality in Cricket Frogs (*Acris crepitans*)

A.L. Reeder,<sup>1</sup> G.L. Foley,<sup>3</sup> D.K. Nichols,<sup>5</sup> L.G. Hansen,<sup>4</sup> B. Wikoff,<sup>4</sup> S. Faeh,<sup>4</sup> J. Eisold,<sup>4</sup> M.B. Wheeler,<sup>1</sup> R. Warner,<sup>2</sup> J.E. Murphy,<sup>4</sup> and V.R. Beasley<sup>4</sup>

<sup>1</sup>Departments of Animal Sciences and <sup>2</sup>Natural Resources and Environmental Sciences, College of Agricultural, Consumer, and Environmental Sciences and <sup>3</sup>Veterinary Pathobiology and <sup>4</sup>Veterinary Biosciences, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL 61801 USA; <sup>5</sup>Department of Pathology, National Zoological Park, Washington, DC 20008 USA

Cricket frogs (*Acris crepitans*) from several different sites in Illinois were collected to assess the effects of environmental contamination on the prevalence of intersex gonads. Of 341 frogs collected in 1993, 1994, and 1995, 2.7% were intersex individuals. There was no statistically significant relationship between the chemical compounds detected and cricket frog intersexuality. However, there was an association approaching significance ( $p = 0.07$ ) between the detection of atrazine and intersex individuals. A comparison of reference sites with sites that had point polychlorinated biphenyl (PCB) and polychlorinated dibenzofuran (PCDF) contamination revealed a significant relationship between sex-ratio reversal and contamination with PCBs and PCDFs. The sex ratio of juvenile frogs studied from three sites with PCB and PCDF point contamination favored males over females, which was the opposite of the sex ratio in control ponds ( $p = 0.0007$ ). The statistically significant correlation between organochlorine contamination and sex-ratio reversal suggests PCBs and PCDFs can influence cricket frog sexual differentiation. The current study suggests that in cricket frogs, sex ratios and the prevalence of intersex gonads are altered by environmental contamination. **Key words:** *Acris crepitans*, amphibian, contaminants, endocrine disrupters, intersexuality, polychlorinated biphenyls, polychlorinated dibenzofurans. *Environ Health Perspect* 106:261–266 (1998). [Online 2 April 1998] <http://ehpnet1.niehs.nih.gov/docs/1998/106p261-266reeder/abstract.html>

The presence of intersex gonads, where an animal is producing gametes of both sexes, has been documented in species ranging from invertebrates (crustaceans) (1) and poikilothermic vertebrates (amphibians, fish) (2–4) to birds (5) and mammals (6), including human beings (7,8). Individuals having both male and female gonads and duct systems have been observed in swine, ranging from 0.03% to 0.53% of the population examined (9–11), but this is rare in other domestic species (12). Intersex gonads have been reported in few wild invertebrate (1) and vertebrate (6) species, and most data sets are inadequate to estimate intersex prevalence. The presence of masculinized and/or feminized tissues does not necessarily impede the mating and copulation process (4,6). However, reproductive function may be impeded by the presence of functional male and female reproductive tissues and an abnormal milieu of hormones (3).

The developmental basis for intersexuality in amphibians involves a sexual bipotentiality of the gonocytes and gonaducts (13). Early amphibian tadpoles have undifferentiated gonads that consist of an outer cortex and an inner medulla (13). These two layers originate from the coelomic epithelium at the medial aspect of the mesonephric kidney in the developing embryo and support the primordial germ cells (14). An indifferent or bisexual state occurs before genetic factors induce sex-determining antigens,

peptides, and hormones that differentiate the cortex into ovarian tissue or medulla into testicular tissue (14,15). A number of environmental factors have been reported to influence sex determination in amphibians. These factors include temperature, pH of the aquatic medium, and presence of chemicals that might pose an insult to the gonads (13). The undifferentiated amphibian gonad is highly sensitive to steroidal compounds, and treatment of males with estrogens during embryonic development can lead to sex reversal or the formation of an ovotestis (14).

Reproductive strategies in amphibians involving sex reversal are rare (4). However, some species of anurans, such as the reed frog (*Hyperolius viridiflavus*), can undergo sex reversal to maximize their lifetime reproductive success under environmental stress and skewed sex ratios (16). Also, some *Bufo* males have vestigial embryonic tissue (Bidder's organ) that can develop into ovarian tissue when the testes are removed or become nonfunctional, but this rarely has been reported in wild populations (4).

A number of mechanisms have been associated with intersexuality. Gonadal intersexuality in certain crustacean species has been attributed to hereditary factors and forms of parasitism (1,17,18). In mammals, gonadal intersexuality appears to be influenced by genetic factors (6,19). Recent studies suggest that environmental

exposure to anthropogenic chemicals that mimic or antagonize the activity of natural estrogens or testosterone may alter sexual development (5,20–23).

The cricket frog (*Acris crepitans*) is a member of the family Hylidae. Hybridization studies have indicated that other hylids have an XY system for sex determination (24). However, no heteromorphic sex chromosomes have been identified in *A. crepitans* based on either size or shape among the karyotypes examined (24). The process of sex determination and the influences on sex determination are not understood for this species. We are unaware of any reports that *A. crepitans* experiences intersexuality or undergoes a sex reversal as a normal part of any of its life stages.

The two studies described in the present report are part of an ongoing research effort investigating risk factors associated with population declines of the northern cricket frog. The goal of this study was to assess the prevalence of gonadal intersexuality in adult and juvenile cricket frogs and to determine whether sexuality is altered in response to exposure to environmental contaminants.

## Materials and Methods

### Study A

**Sites and cricket frog specimens.** In 1993, a pilot study focused on site identification and preliminary assessment of histologic lesions in Illinois cricket frog populations. Twenty sites were identified. For 1994 and 1995, pond sites were chosen based on specific criteria, including the presence of cricket frogs and suitable habitat. Suitable habitat included gently sloping banks with low-lying terrestrial plants that merged with shallow water containing abundant aquatic macrophytes. In the latter 2 years, an equal number of phenotypic male and female frogs

Address correspondence to V.R. Beasley, Department of Veterinary Biosciences, College of Veterinary Medicine, University of Illinois, 2001 S. Lincoln Avenue, Urbana, IL 61801-6199 USA.

We are grateful to Laura Hungerford for help with the statistical analysis and indebted to Lauren Brown for his continuing input. We thank the Midwest Society of Toxicology and the John G. Shedd Aquarium for partial funding of this study. Received 22 July 1997; accepted 6 January 1998.

were collected at each site. In 1994, eight sites in Illinois with ponds of similar size were studied, including five in the southern part of the state, two in the central area, and one in the northeast part of the state. In 1995, seven of the ponds studied in 1994, as well as a pond in an agricultural area of east-central Illinois, were studied.

Cricket frogs were captured along pond banks with hand-held fish nets. To capture equal numbers of both sexes, we preliminarily sexed adult cricket frogs by examining for the presence or absence of a vocal pouch, a secondary sex characteristic of males. This pouch is absent in juveniles, which were caught later in the season. Specimens were

killed in a solution of 3-aminobenzoic acid ethyl ester methanesulfonate salt (MS-222; Sigma Chemical Company, St. Louis, MO) in water (5:100 weight to volume). In 1993, gonads of 50 intact cricket frogs from 18 sites in east-central and southern Illinois were grossly examined. In 1994, after the frogs were killed, half were placed in 10% neutral buffered formalin (NBF) and the other half were frozen. In 1995, three-fourths of the frogs were placed in NBF and one-fourth were frozen. In 1994 and 1995, we dissected the gonads from both the fixed and frozen frogs using a dissecting microscope. At that time, frogs were sexed by gross examination of the gonads. In 1994, gonads from 242 frogs were examined, with a range of 24–38 individuals from the various sites. In 1995, gonads from 40 frogs were examined, with a range of 4–7 per site.

For 1993 specimens only, whole-body demineralization was achieved with formic acid–sodium citrate decalcifying solution, and whole-body histologic sections were prepared. Frozen gonads from 1994 and 1995 were thawed in NBF. Fixed gonads and frozen-fixed gonads were then embedded in paraffin, sectioned at 5  $\mu$ m, and stained with hematoxylin and eosin. Sections were examined with a light microscope and assigned sex based on histologic examination. We defined intersex individuals as those frogs that had either an ovotestis (a gonad that contains both ovarian and testicular tissue) or that had one ovary and one testis.

**Contaminant assays.** Composite water and sediment samples were collected in acetone-rinsed containers from multiple locations in each pond studied during 1994 and 1995. Specimens were collected twice, at the end of May and late July/early August in 1994, and once in 1995, at the end of May.

For sites 11 and 12, an additional set of specimens was collected in late June 1998, when a severe aquatic plant die off was observed (this was an unscheduled sampling). Each sample was frozen and sent to the State of Illinois Animal Disease Laboratory at Centralia for analysis. Water and sediment samples were analyzed for herbicides, fungicides, insecticides, polychlorinated biphenyls (PCBs), and metals (Table 1). Water samples (900 ml) were partitioned three times with methylene chloride (50 ml each time): once with no pH modification, a second time after adjusting the pH to >11.0 with 50% sodium hydroxide in water, and a third time after adjusting the pH to <2.0 with sulfuric acid. The three methylene chloride extracts were combined, mixed with 0.1 ml decane, and evaporated to near dryness at no higher than 35°C. Sediment samples were extracted in acetonitrile:water (9:1), sonicated for 1 hr, filtered, and dried. For herbicide analyses, the residue from either water or sediment was resolubilized in acetonitrile:water (9:1) and analyzed by HPLC with UV detection at 220 nm. For insecticides and PCBs, residues were solubilized in isooctane and analyzed by gas chromatography with either electron capture detection (organochlorine insecticides and PCBs) or nitrogen–phosphorus detection (organophosphorus and carbamate insecticides) (Hewlett Packard HP5890, Series 2, Palo Alto, CA). Water and soil samples for lead analysis were digested with concentrated nitric acid, filtered, and assayed by atomic absorption spectrophotometry with a graphite furnace. Sediment samples for mercury analysis were analyzed after digestion in nitric acid and subsequent treatment with nitric acid, hydroxylamine hydrochloride, and stannous chloride. A Perkin Elmer 4000 atomic absorption spectrophotometer

**Table 1.** Compounds analyzed in water and sediment samples (water detection limits in parentheses,  $\mu$ g/l)

Herbicides	Insecticides
Alachlor (5)	Carbamates
Ametryn (1)	Aldicarb (10)
Atrazine (0.5)	Aminocarb (5)
Barban (5)	Bendiocarb (5)
Bifenox (5)	Carbaryl (5)
Bromacil (1)	Carbofuran (5)
Butachlor (5)	Lannate (5)
Butylate (5)	Methiocarb (5)
Chloroprotham (5)	Oxamyl (5)
Chlorothal (5)	Organochlorine
Cyanazine (0.5)	Aldrin (0.5)
Diclofop (5)	Chlordane (0.5)
Dinitramine (5)	DDD (0.5)
Dipropetryn (5)	DDE (0.5)
Diuron (5)	DDT (0.5)
EPTC (5)	Dieldrin (0.5)
Fluchloralin (5)	Endosulfan (0.5)
Hexazinone (5)	Endrin (0.5)
Linuron (5)	Heptachlor (0.5)
Metolachlor (5)	Heptachlor epoxide (0.5)
Metribuzin (5)	Lindane and isomers (0.5)
Monuron (5)	Methoxychlor (0.5)
Napropamide (5)	Mirex (0.5)
Naptalam (5)	PCBs (0.5)
Oryzalin (5)	Organophosphorous
Pebulate (5)	Chlorpyrifos (1)
Pendimethalin (5)	Diazinon (1)
Profluralin (5)	Dimethoate (1)
Prometon (5)	Disulfoton (1)
Prometryn (5)	Ethoprop (1)
Propanil (5)	Ethyl parathion (1)
Propazine (5)	Fenchlorphos (1)
Propham (5)	Fenthion (1)
Simazine (1)	Fonofos (1)
Terbuthylazine (5)	Isofenphos (1)
Terbutryn (5)	Malathion (1)
Trifluralin (5)	Methodathion (1)
	Methyl parathion (1)
	Mevinphos (1)
	Phorate (1)
	Terbufos (1)
	Trichlorfon (1)
	Fungicides
	Benomyl (5)
	Hexachlorobenzene (0.5)
	Thiobencarb (10)
	Metals
	Lead (5, 0.1 mg/kg <sup>a</sup> )
	Mercury (0.1 $\mu$ g/kg <sup>a</sup> )

<sup>a</sup>Sediment detection limits.

**Table 2.** Differences in PCB contamination among the four cricket frog collection sites at Crab Orchard Lake (see Fig. 2)

Site	PCB (mg/kg wet weight)		Volume above target <sup>c</sup> (cubic yards)	
	Inert soil <sup>a</sup>	Sediment	Soil	Sediment
Site C (Control) Shallow Bay	<0.5	ND	ND	ND
Site D (Job Corps) Landfill Pond	2–50,000	0.9–260	4,307	3,440
Area 9 Landfill	<69,000	11.16	76,388	17,363 <sup>d</sup>
E Shallow Bay		4.1–18	ND	
F Shallow Bay		0.21–4.1	0.81	

ND, no data.

<sup>a</sup>PCB concentration ranges identified by the EPA (25) and used to establish remediation necessity as well as planned remedial actions.

<sup>b</sup>Whole-body PCB concentrations for white-footed mice (*Peromyscus leucopus*) and southern leopard frogs (*Rana pipiens sphenoccephala*) collected from the respective sites (26).

<sup>c</sup>Volumes of soils and sediments scheduled to be excavated and incinerated at the target sites to achieve dry weight PCB concentration of <1 mg/kg (upper 30.5 cm of soil), <25 mg/kg (subsurface soil), or <0.5 mg/kg (sediment). Soils and sediments containing PCBs at <25 mg/kg are not included in volumes because they are scheduled to be used as backfill (27).

<sup>d</sup>Combined value from both shallow bay sites E and F.

(Norwalk, CT) was used, and for mercury, a cold vapor mercury analysis kit was used.

## Study B

On 18 and 24 July 1995, 16 juvenile cricket frogs were collected from each of three sites in the Crab Orchard National Wildlife Refuge near Carbondale in southern Illinois, where point contamination with PCBs, polychlorinated dibenzofurans (PCDFs), and polychlorinated dibenzo-*p*-dioxins (PCDDs) previously had occurred (25–28). On the same days, 16 juveniles also were collected from each of three control sites located outside the area of PCB and PCDF contamination. One of the control sites was inside the Crab Orchard refuge, and the other two were located approximately 16 km from the refuge. Extents of PCB contamination from the Crab Orchard sites are compared in Table 2. Methods for cricket frog collection, euthanasia, tissue fixation, histologic preparation, and examination were as described above.

## Statistical Methods

Fisher's Exact test was used to compare prevalence of intersex gonads at study sites and detection of atrazine at those sites. Fisher's Exact test was also used to compare sex ratios among control and PCB/PCDF sites. The Sigma Stat (29) statistical software package was used for the analyses. A value of  $\alpha = 0.05$  was chosen to detect significant differences.

## Results

### Study A

Testicular tissue of *A. crepitans* generally is heavily pigmented, but approximately 20% of males had testes with reduced or absent pigmentation. When this phenomenon was present, it was noted most often on the right side. Normal spermatogenesis was observed in pigmented and nonpigmented testes. Mature cricket frog ovaries of normal females contained large egg masses, at times distending the body cavity, with bipolar eggs (tan/black coloring) that could be seen without a microscope. Immature egg masses could be readily identified, but the oocytes therein were seen only on the microscopic level. In specimens that had a testis on one side and an ovary on the other, ovarian size ranged from well-developed mature female size (with abdominal distention associated with the egg mass) to extremely small, with as few as five oocytes present.

Gonadal sexuality data are presented in Table 3. Of the 55 adult and juvenile male and female cricket frogs from 1993, two individuals had both an ovary and testis. In

the testis of one of these individuals, spermatogenesis was normal. In the other individual, an immature ovary was present, as well as a testis with no active spermatogenesis. This corresponds to an intersex prevalence (IP) of 3.6% for the year 1993. In 1994, of 243 cricket frogs examined, 6 specimens from five sites exhibited intersex characteristics, for an overall IP of 2.5%. Five of the affected individuals had areas of normal spermatogenesis interspersed with oocytes (ovotestes). One of the frogs had a mature ovary and a mature testis with normal spermatogenesis. In 1995, only one intersex individual was identified from the 43 frogs examined, corresponding to an intersex prevalence of 2.3%. This individual had an ovotestis (Fig. 1). For specimens from all 3 years, the IP was 2.6%.

Water analysis results are presented in Table 4. In 1994, of five sites where intersex individuals were found, four had detectable atrazine. In contrast, of the four sites in which no intersex frogs were found, only one contained detectable levels of atrazine. The Fisher's Exact test of the relationship between detection of atrazine and the presence of one or more intersex cricket frogs approached significance ( $p = 0.07$ ). At the single site treated in 1994 with copper sulfate, 1 cricket frog of the 33 collected had an ovotestis. In 1995, no relationship between detection of atrazine and intersex was apparent. No intersex individuals were identified among the three frogs collected from the pond treated with endothall in 1995. Concentrations of lead from both years were considered background and not associated with intersexuality.

**Table 3.** Cricket frog intersexuality prevalence for 1993, 1994, and 1995 by year and site

Year	Site	No. of intersexes	Intersex condition	Frogs per site	Site prevalence (%)	Other abnormalities
1993 <sup>a</sup>	1	0		2	0	None
	2	0		1	0	None
	3	0		1	0	None
	5	0		3	0	None
	6	0		2	0	None
	8	0		1	0	None
	10	0		1	0	None
	11	0		5	0	None
	12	1	Ovary and testis	5	20	None
	13	0		5	0	None
	14	0		5	0	None
	15	0		4	0	None
	16	1	Ovary and testis	5	20	None
	17	0		5	0	None
	18	0		5	0	None
	19	0		5	0	None
	All	2		55	3.6	
1994	5	2	Ovotestes	35	5.8	Decreased testicular pigmentation
	11	1	Ovotestes	25	4.3	Reduced testes size One frog had 3 forelimbs
	12	1	Ovary and testis	39	2.6	One frog had 3 testes
	15	0		32	0	None
	16	1	Ovotestes	33	3.0	Head kidney present
	19	0		33	0	None
	20	1	Ovotestes	24	4.2	None
	22	0		5	0	None
	23	0		17	0	None
	All	6		243	2.5	
1995	4	0		7	0	None
	5	0		5	0	None
	11	0		6	0	None
	12	0		4	0	None
	16	0		5	0	None
	19	0		4	0	None
	20	1	Ovotestes	4	25	Decreased testicular pigmentation
	23	0		3	0	None
	24	0		1	0	None
	25	0		4	0	None
	All	1		43	2.3	
1993–1995 <sup>b</sup> All		9		341	2.6	

<sup>a</sup>Whole-body sectioning performed.

<sup>b</sup>Total frogs examined from 3 years and combined prevalence.



## Study B

In frogs collected from the PCB point contaminated sites and control sites, only one intersex individual was found. This frog was from one of the control sites and had an ovotestis with areas of normal spermatogenesis and oocytes. The sex ratios of juvenile frogs differed significantly between PCB/PCDF contaminated and control sites (Fig. 2). In 13 juveniles from the contaminated sites and 13 from the control sites,

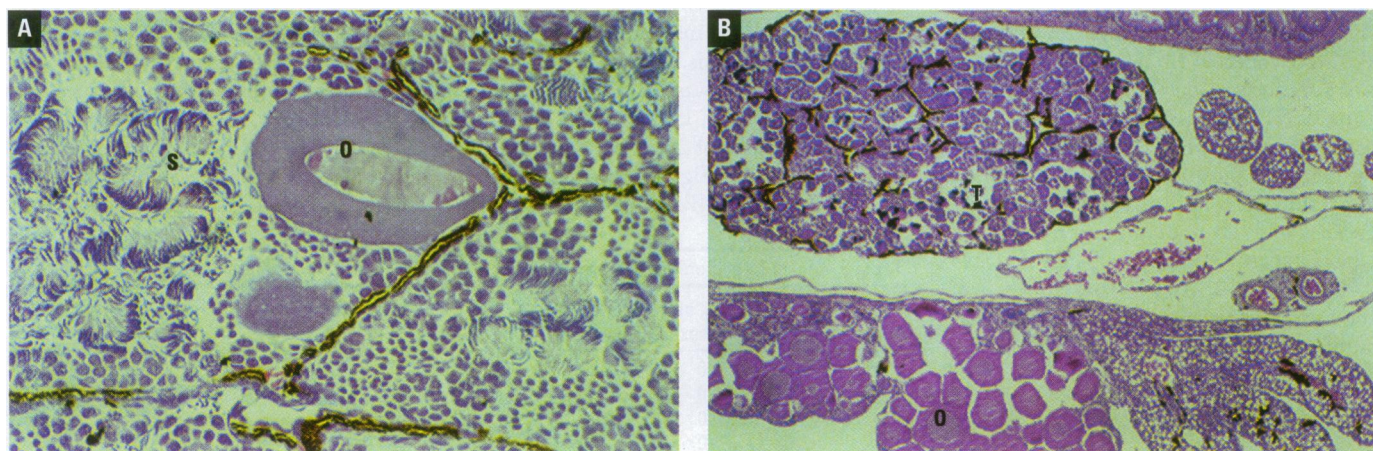
gonadal tissue was immature and could not be identified for histologic preparation. The Fisher's Exact test used to examine the association between sex ratios of the PCB/PCDF contaminated and control groups revealed a significant difference ( $p = 0.0007$ ).

## Discussion

Ovotestes have been noted previously in other species of anurans (13); however, to our knowledge, this is the first study to investigate

the prevalence of intersexuality in any species of amphibian or to examine potential influences of contaminants on sexuality of wild amphibians.

The overall prevalence of intersexuality in study A cricket frogs from 1993 to 1995 was 2.6%. This may be consistent with the natural prevalence of intersexuality or may represent a prevalence altered by hormonally active environmental contaminants. A number of insecticides formerly used in



**Figure 1.** Photomicrographs of two forms of intersexuality in cricket frogs. (A) Oocytes (O) mixed in with the seminiferous tubules; adjacent tubules had normal spermatogenesis (S). (B) The frog had one ovary (O) and one testis (T) with normal spermatogenesis.

**Table 4.** Water and sediment contamination results<sup>a</sup>

Year	Site	Matrix <sup>b</sup>	Pesticide	Pesticide concentration (mg/l)			Lead matrix <sup>c</sup>	Lead concentration (mg/l)			Other pesticides
				May	June	July/Aug		May	June	July/Aug	
1994	5	Water	Atrazine	ND		0.003	Sediment	9.12		12.67	
	11 <sup>d</sup>	Water	Metolachlor	0.002	0.001	0.002	Sediment	12.18	13.3	ND	
			Atrazine	ND	ND	0.006					
		Sediment	Metolachlor	0.04	ND	ND					
			Atrazine	0.07	0.04	ND					
	12 <sup>d</sup>	Water	Chlorpyrifos	3.1 <sup>e</sup>	ND	ND	Water	11.5 <sup>e</sup>	ND	ND	
		Water	Atrazine	ND	0.002	ND	Sediment	11.72	ND	8.86	
	15						Water	8.2 <sup>e</sup>			
							Sediment	7.78		6.86	
	16						Water	7.2 <sup>e</sup>			Copper sulfate <sup>f</sup>
							Sediment	9.52		12.36	
	19						Water	7.3 <sup>e</sup>			
							Sediment	10.34		8.74	
20	Water	Atrazine	ND		0.003	Sediment	7.42		8.9		
23						Sediment	16.92		4.4		
1995	4	Water	Atrazine	0.015			Sediment	8.5			Copper sulfate <sup>f</sup>
			Cyanazine	0.017							
	5						Sediment	4.0			
	11	Water	Atrazine	0.002			Sediment	9.8			
	12	Water	Atrazine	0.001			Sediment	4.0			
	16						Sediment	13.0			Copper Sulfate <sup>f</sup>
	19						Sediment	9.3			
	20						Sediment	12.2			
	23						Sediment	3.4			Endothall <sup>f</sup>
	24						Sediment	6.85			
	25						Sediment	1.41			

ND, not detected.

<sup>a</sup>In 1994, water and sediment were sampled twice; at the end of May and end of July–early August. In 1995, water and sediment were sampled one time; at the end of May.

<sup>b</sup>Pesticides were measured on a wet weight basis.

<sup>c</sup>Sediment lead was measured on a dry weight basis.

<sup>d</sup>These sites were sampled an extra time.

<sup>e</sup>Units are µg/l.

<sup>f</sup>Pesticides used by land owners, but not included in the analytical profile.



midwestern agriculture including DDT, dieldrin, and endosulfan have demonstrated estrogenic potential (5,21–23,30,31), although none of these was present at detectable concentrations in our study ponds. In contrast, the herbicide atrazine, which often was detected, possesses limited estrogenic potential (31). Our 1994 data suggested a relationship between environmental contamination with atrazine and the prevalence of intersexuality in cricket frogs. This was not apparent in 1995, but fewer frogs were collected from each site that year, substantially reducing statistical power. Atrazine-contaminated sites may represent areas of great agricultural impact, potentially involving other chemical, physical, and biotic factors.

Sex ratios of cricket frogs shortly after metamorphosis strongly favor females (32), as was the case at the control sites in study B. However, at the sites contaminated by PCBs and PCDFs, there was a striking sex-ratio reversal in juvenile cricket frogs resulting in a high number of males. As previously documented in cricket frog populations in Kansas, the percentage of female cricket frogs declines from metamorphosis to breeding (32), possibly due to greater predation rates as they become laden with eggs. A marked increase in the male:female sex ratio may decrease the likelihood of successful mating and may represent a biomarker

for contamination with hormonally active contaminants.

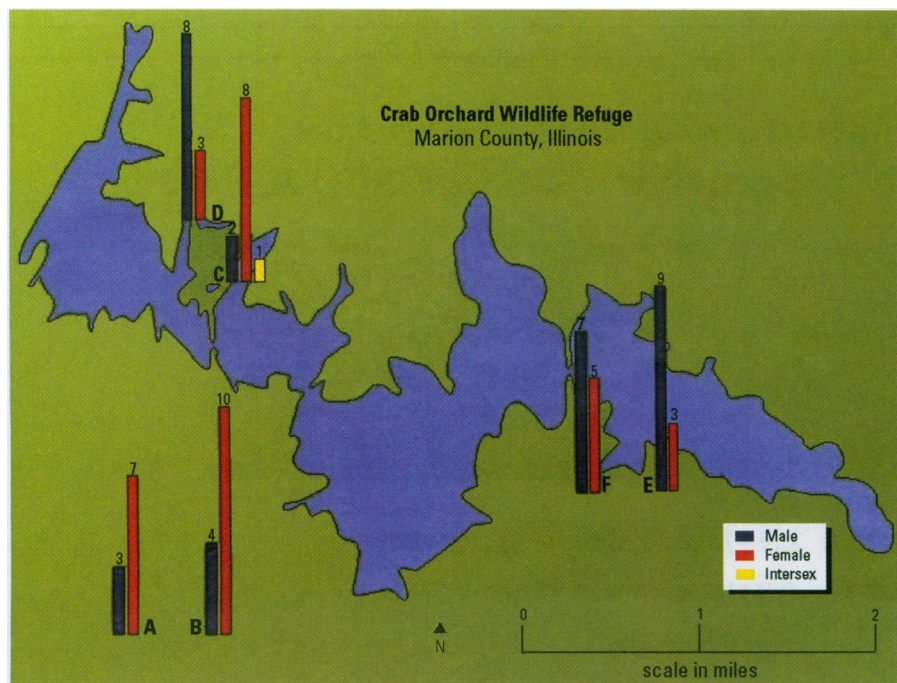
Feminization due to estrogenic and/or antiandrogenic contaminants has been well documented in many species of vertebrates (5,20–23,30–34). Various congeners and mixtures of PCBs, PCDFs, and PCDDs possess a spectrum of estrogenic, antiestrogenic, and antiandrogenic effects (27, 34–38). Soil, dust, and air near the contaminated sites contained both estrogenic and antiestrogenic PCBs and PCDFs, but antiestrogenicity dominates the net effect of the PCB/PCDF mixtures at these sites (28,36). Although the antiestrogenic coplanar compounds were present at lower concentrations than the estrogenic compounds, the former are more potent, and estrogenic effects are unmasked only after coplanar compounds are stripped from the mixtures (36). Dose–response relationships for PCBs and PCDFs may be nonlinear and therefore challenging to characterize experimentally. For example, such compounds may cause low-dose effects that are absent at higher doses (37,38). In addition, enzyme induction at higher doses may alter toxicokinetics of these contaminants, thereby decreasing concentrations at receptors (29,36,37). Furthermore, all types of congeners present at the contaminated sites of Crab Orchard, as well as their mixtures, disrupt thyroid hormone ( $T_4$ ) function.

Reduced thyroid hormonal activity may add complexity to interactive effects. As PCB dose increases and systemic  $T_4$  declines, some rodent tissues may be less responsive to estrogenlike actions (36,37), and  $T_4$  is critical to amphibian metamorphosis (15). Because of such factors, it is difficult to predict the net actions of complex environmental mixtures of hormonally active contaminants.

Whether genetics, temperature, or a combination of these determines sex in cricket frogs remains to be investigated. Witschi (39) reported that tadpoles of *Rana temporalis* in water at 10°C and lower produced 100% females, while 15–21°C produced a near 1:1 ratio of the two sexes, and tadpoles at 27°C or higher produced 100% males at metamorphosis. Hayes and Licht (40) found that, unlike *Xenopus laevis*, *Bufo boreas* metabolized testosterone more efficiently at higher temperatures than at lower temperatures. Rates of conversion of testosterone to estradiol were not determined. Because of the many enzyme systems involved, the roles of temperature and contaminants including PCBs and PCDFs on enzymes involved in steroid production and metabolism are likely to be complex (41). Although we did not monitor water temperatures, there is no reason to suspect marked differences among the water temperatures of the various sites. Thus, our study strongly suggests an association between PCB/PCDF contamination and sex-ratio reversal in cricket frogs. Whether atrazine accounts for findings of intersexuality is less clear. Understanding genetic mechanisms of sex determination in this species, coupled with controlled exposures to PCBs, PCDFs, atrazine, and other contaminants, is warranted to determine mechanisms by which genetic and/or temperature-related determinants of sex are altered by exposure to anthropogenic chemicals.

## REFERENCES

- Hough AR, Bannister NJ, Naylor E. Intersex in the mysid *Neomysis integer*. *J Zool Soc Lond* 226:585–588 (1992).
- Bortone SA, Davis WP. Fish intersexuality as indicator of environmental stress. *Bioscience* 144:165–171 (1994).
- Frye FL, Miller H. Hermaphroditism in a tomato frog (*Dyscophus antongilii*). *J Zoo Wildl Med* 25:154–157 (1994).
- Sullivan BK, Propper CR, Demlong MJ, Jarvey LA. Natural hermaphroditic toad. *Copeia* 2:470–472 (1996).
- Fry DM, Toone CK. DDT-induced feminization of gull embryos. *Science* 213:922–924 (1981).
- Jimenez R, Burgos M, Sanchez A, Sinclair AH, Alarcon FJ, Marin JJ, Ortega E, Diaz de la Guardia R. Fertile females of the mole *Talpa occidentalis* are phenotypic intersexes with ovotestes. *Development* 118:1303–1311 (1993).
- Givens JR, Wiser WL, Summitt RL, Kerber IJ, Andersen RN, Pittaway DE, Fish SA. Familial male

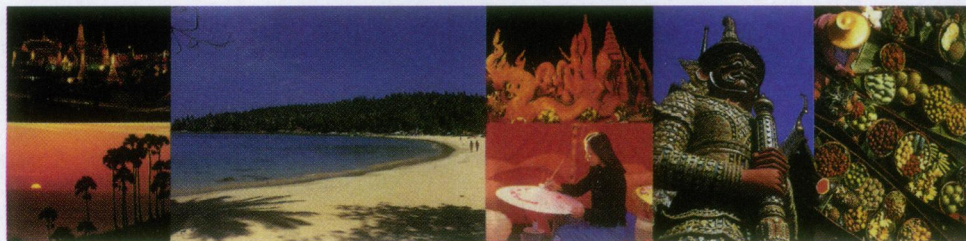


**Figure 2.** Map of Crab Orchard Lake illustrating sex-ratio reversal in PCB/PCDF-contaminated sites compared to controls. Sites A, B, and C were control sites. Sites A and B were two small ponds located approximately 16 km north-northwest of the lake. Site C was located on the lake. Sites D, E, and F were PCB/PCDF-contaminated sites. Site D was a small pond adjacent to the lake. Sites E and F were located near the Sangamo Landfill on the lake. There was a significant correlation between sex-ratio reversal and PCB/PCDF contamination ( $p = 0.0007$ ). Numbers above bars signify  $n$  for the groups.



- pseudohermaphroditism without gynecomastia due to deficient testicular 17-ketosteroid reductase activity. *N Engl J Med* 291:938-944 (1974).
8. Walsh PC, Madden JD, Harrod MJ, Goldstein JL, MacDonald PL, Wilson JD. Familial incomplete male pseudohermaphroditism, type 2. Decreased dihydrotestosterone formation in pseudovaginal perineoscrotal hypospadias. *N Engl J Med* 291:944-949 (1974).
  9. Albertsen K. Hermafroditisme hos svin. *Nordic Vet Med* 3:849-868 (1951).
  10. Pfeffer AH, Winter H. Hermaphrodites in Australian pigs, occurrence and morphology in an abattoir survey. *Aust Vet J* 53:153-162 (1977).
  11. Krishnamurthy S, Macpherson JW, King GJ. Intersexuality in Ontario swine. *Can J Anim Sci* 5:807-815 (1971).
  12. McEntee K. Reproductive Pathology of Domestic Mammals. New York:Academic Press, 1990;8-30.
  13. Foote CL. Intersexuality in amphibians. In: *Intersexuality in Vertebrates Including Man* (Armstrong CN, ed). New York:Academic Press, 1964;234-272.
  14. George FW, Wilson JD. Sex determination and differentiation. In: *The Physiology of Reproduction*, (Knobil E, Neill JD, eds). 2nd ed. New York:Raven Press, 1994;3-28.
  15. Noble GK. The Biology of the Amphibia. New York:Dover Publications, 1931.
  16. Grafe TU, Linsenmair KE. Protogynous sex change in the reed frog (*Hyperolius viridiflavus*). *Copeia* 4:1024-1029 (1989).
  17. Korczyński RE. Additional records on intersexuality in the isopod *Mesidotea sibirica* (Birula, 1986). *Can J Zool* 66:1896-1898 (1988).
  18. Dunn A, Adams MJ, Smith JE. Is intersexuality a cost of environmental sex determination in *Gammarus duebeni*? *J Zool Soc London* 231:383-389 (1993).
  19. Hunter RHF. Sex Determination, Differentiation, and Intersexuality in Placental Mammals. New York:Cambridge University Press, 1995.
  20. White R, Jobling S, Hoare SA, Sumpter JP, Parker MG. Environmentally persistent alkylphenolic compounds are estrogenic. *Endocrinology* 135:175-182 (1994).
  21. Arnold SF, Klotz DM, Collins BM, Vonier PM, Guillette LJ, McLachlan JA. Synergistic activation of estrogen receptor with combinations of environmental chemicals. *Science* 272:1489-1492 (1996).
  22. Guillette LJ Jr, Gross TS, Masson GR, Matter JM, Percival HF, Woodward AR. Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. *Environ Health Perspect* 102:680-688 (1994).
  23. Kelce WR, Stone CR, Laws SC, Gray LE, Kemppainen JA, Wilson EM. Persistent DDT metabolite *p-p'*-DDE is a potent androgen receptor agonist. *Nature* 375:581-585 (1995).
  24. Anderson K. Chromosome evolution in holoarctic Hyla tree frogs. In: *Amphibian Cytogenetics and Evolution* (Green DM, Sessions SK, eds). New York:Academic Press, 1991;305-321.
  25. U.S. EPA. Superfund record of decision: Sangamo/Crab Orchard NWR (USD01) IL. PB91-921509. Washington:U.S. Environmental Protection Agency, 1991.
  26. McKee MJ. Ecotoxicological evaluation of Area 9 Landfill at Crab Orchard National Wildlife Refuge: Biological impact and residues. Champaign, IL: Hazardous Waste Research and Information Center, 1992.
  27. IT Corporation. Pre-design Contamination Delineation Report for the PCB Operable Unit at Crab Orchard National Wildlife Refuge, Marion, Illinois. Knoxville, TN: International Technologies Corporation, 1993.
  28. Li M-H, Hansen LG. Enzyme induction and acute endocrine effects in prepubertal female rats receiving environmental PCB/PCDF/PCDD mixtures. *Environ Health Perspect* 104:712-722 (1996a).
  29. Turke TM, Schwallie-Farmer C, Rubenstein S, Youtz P. SigmaStat. San Rafael, CA: Jandel Scientific (1994).
  30. Kupfer D, Bulger WH. Estrogenic properties of DDT and its analogs. In: *Proceedings of the Symposium on Estrogens in the Environment*, 10-12 Sept 1979, Raleigh, NC. New York:Elsevier/North Holland, 1980;239-263.
  31. Eldridge JC, Tennant MK, Wetzel LT, Breckenridge CB, Stevens JT. Factors affecting mammary tumor incidence in chlorthiazine-treated female rats: hormonal properties, dosage, and animal strain. *Environ Health Perspect* 102(suppl 11):29-36.
  32. Burkett RD. An ecological study of the cricket frog, *Acris crepitans*. *Vert Ecol Syst* (special publication) 10:89-103 (1984).
  33. Newbold RR, McLachlan JA. Diethylstilbestrol associated defects in murine genital tract development. In: *Estrogens in the Environment II* (McLachlan JA, ed). Amsterdam:Elsevier Science, 1985;288-318.
  34. Mabry TA, Bjerke DL, Moore RW, Fendron-Fitzpatrick A, Peterson RE. *In utero* and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol Appl Pharmacol* 114:118-126 (1992).
  35. Jansen HT, Cooke PS, Porcelli J, Liu T-C, Hansen LG. Estrogenic and antiestrogenic actions of PCBs in the female rat: *in vitro* studies. *Reprod Toxicol* 7:237-248 (1993).
  36. Li M-H, Hansen LG. Responses of prepubertal female rats to environmental PCBs with high and low dioxin equivalencies. *Fundam Appl Toxicol* 33:282-293 (1996).
  37. Li M-H, Zhao Y-D, Hansen LG. Multiple dose toxicokinetic influence on the estrogenicity of 2,2',4,4',5,5'-hexachlorobiphenyl. *Bull Environ Contam Toxicol* 53:583-590 (1994).
  38. Li M-H, Rhine C, Hansen LG. Hepatic enzyme induction and acute endocrine effects of 2,3,3',4',5-pentachlorobiphenyl in prepubertal female rats. *Archiv Environ Contam Toxicol* (in press).
  39. Witschi E. Development of Vertebrates. Philadelphia: W.B. Saunders, 1956;191-193.
  40. Hayes TB, Licht P. Factors influencing testosterone metabolism by anuran larvae. *J Exp Zool* 271:112-119 (1995).
  41. Crews D, Bergeron JM. Role of reductase and aromatase in sex determination in the red-eared slider (*Trachemys scripta*), a turtle with temperature-dependent sex determination. *J Endocrinol* 143:279-289 (1994).

## THIRD INTERNATIONAL CONFERENCE ON ENVIRONMENTAL MUTAGENS IN HUMAN POPULATIONS



**NOVEMBER 29-DECEMBER 4, 1998**  
**BANGKOK • AYUDHAYA • KHAO YAI**  
**THAILAND**

Dr. Malyn Chulasiri  
Faculty of Pharmacy, Mahidol University  
Sri-Ayudhaya Road, Bangkok 10400, Thailand

Phone: (662) 644-8692  
Fax: (662) 247-4696, 247-9428  
E-mail: pymcl@mahidol.ac.th